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Thiopurine Methyltransferase Deficiency in Childhood Lymphoblastic Leukaemia: 6-Mercaptopurine Dosage Strategies

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Daily 6-mercaptopurine (6MP) forms the backbone of continuing chemotherapy for childhood lymphoblastic leukaemia (ALL). A major metabolic route is catalysed by thiopurine methyltransferase (TPMT). TPMT deficiency occurs in 1 in 300 individuals and results in high concentrations of thioguanine nucleotides (TGNs), cytotoxic 6MP metabolites. A leukaemic child taking 6MP repeatedly developed profound pancytopenias. TPMT deficiency was confirmed. TGN formation was then studied on attenuated 6MP dosages. Four weekly oral doses of 75 mg/m^2 6MP produced TGNs of 2348 pmol/8 × 10^8 red cells, nearly double the maximum TGNs recorded in ALL children with TPMT activity taking long term daily 75 mg/m^2 6MP. Grossly elevated TGN concentrations were also produced at 10% standard 6MP dosage (7.5 mg/m^2 daily), accompanied by unacceptable 6MP toxicity (neutropenia, diarrhoea, vomiting). The child was eventually stabilised on 10% alternate day therapy and after 15 weeks TGNs were 1670 pmol, just above the upper end of the TGN range for ALL children with TPMT activity. Med. Pediatr. Oncol. 29:252–255, 1997.

Key words: leukemia; 6-mercaptopurine; thiopurine methyltransferase; toxicity

INTRODUCTION

A two-year period of 6-mercaptopurine (6MP) chemotherapy is an integral component of the current UK trial for childhood ''acute'' lymphoblastic leukaemia (ALL). The ability of a child to form adequate amounts of drug derived 6-thioguanine nucleotides (TGNs) at a standard dose of 6MP is clinically important in terms of relapse-free survival [1]. Thiopurine methyltransferase (TPMT) catalysed 6MP *S*-methylation competes with TGN formation.

The activity of TPMT, in all cells and tissues, is regulated by a common genetic polymorphism [2], and TPMT activity within the red blood cell (RBC) is significantly correlated with leukaemic blast TPMT activity in children with ALL [3]. TPMT deficiency occurs in 1 in 300 individuals who are homozygous for the *TPMT*^{L} allele [4], whilst 11% of the population have intermediate TPMT activity (heterozygous *TPMT*^{L}/*TPMT*^{H}), and 89% have high TPMT activity (homozygous *TPMT*^{H}). Congenital deficiency is associated with grossly elevated TGN concentrations and profound myelosuppression on exposure to 6MP. This problem has been documented in adults on immunosuppressive treatment with the 6MP pro-drug azathioprine [5,6] and described in children with ALL [7,8,9]. The aim of this study was to assess TGN formation at attenuated 6MP dosages in a TPMT deficient child.

MATERIALS AND METHODS

Patient and Therapy

A 9-year-old girl with ''common'' ALL was treated according to the UK ALL protocol XI [10]. She entered remission and her progress was uneventful until the continuing phase of chemotherapy. Continuing chemotherapy (protocol week 8 to week 100) includes daily 6MP at 75mg/m^2 and weekly methotrexate at 20 mg/m^2 (100% protocol doses), with protocol defined cytopenia driven dosage reductions; when blood cell counts recover above threshold a protocol-directed cycle of parallel dose increments follows. In addition, intravenous vincristine (1.5 mg/m^2, monthly) and a monthly pulse of 5 days of oral prednisolone (40 mg/m^2), are given irrespective of blood cell counts. 6MP was tolerated for 10 days at 100% when she developed neutropenia (neutrophils 0.32 × 10^9/l) and thrombocytopenia (platelet count 80 × 10^9/l).

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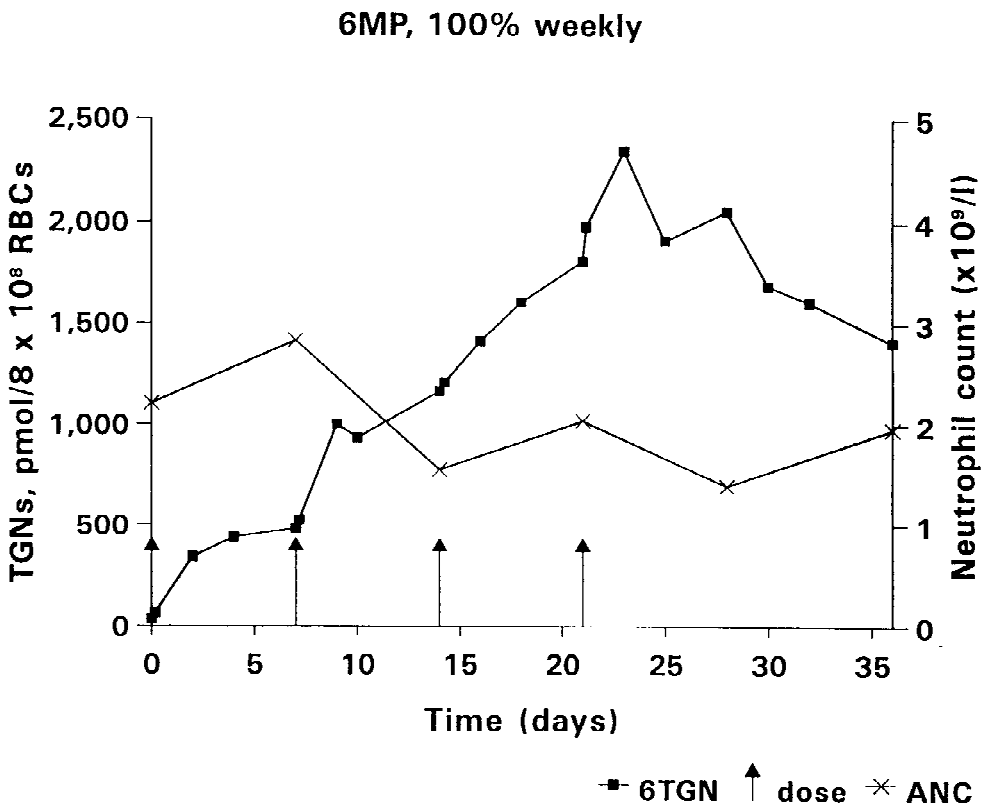

Fig. 1. The accumulation of RBC TGNs during the 100% (75mg/m^2) weekly dosage study. 6MP was taken, as indicated by the large arrows, on days 0, 7, 14, and 21. The Figure illustrates the neutrophil (ANC) counts on days 0, 7, 14, 21, 28, and 36. The platelet counts on those days were 170, 287, 170, 164, 126, and 193 × 10^9/l respectively. The 10% daily 6MP study followed, starting at day 36.

Seven weeks later her counts recovered and antimetabolite therapy was recommenced at 50% and withdrawn 14 days later (platelets 20 × 10^9/l). On count recovery, five weeks later, the child was rechallenged with 50% 6MP which was tolerated for only 14 days. Four weeks later counts recovered, daily 6MP was again restarted at reduced dosage (10% × 2 weeks followed by 25% × 2 weeks), and withdrawn due to pancytopenia.

A blood sample was obtained at the time of 6MP withdrawal for the measurement of TGNs (2599 pmol/8 × 10^8 RBCs) [11]. Blood samples obtained 2, 3, 4, and 5 weeks later contained 1038, 562, 424, 282 pmol TGNs/8 × 10^8 RBCs respectively. The initial TGN half-life ($t½_\alpha$, 0–3 weeks post-dose) was 12 days and $t½_\beta$ (3–5 weeks post-dose) 25 days. The degree of 6MP sensitivity and the formation of grossly elevated TGNs suggested functional TPMT deficiency. A blood sample obtained after 6MP withdrawal and taken 6 weeks post blood transfusions gave < 0.74 units TPMT/ml RBCs, below the lower limit of the assay used [12].

Monthly vincristine and oral prednisolone were given as stated in the UK ALL XI trial [10], as was oral methotrexate i.e. the weekly methotrexate dosage was adjusted in parallel to the 6MP dose. In total, antimetabolite therapy was withdrawn for 75% of the first 34 weeks of scheduled 6MP chemotherapy. Attenuated 6MP dosages were studied after recovery of the blood cell counts above threshold following a 10-week episode of neutropenia. At the start of the study the protocol directed methotrexate dose was 10 mg/m^2 weekly (50% protocol), this was maintained throughout the study period.

Study Design

6MP was studied at two dosages, 100% weekly and 10% daily. Each schedule was given for 4 weeks with a one week ''wash-out'' for the calculation of TGN half-lives. Full blood cell counts were obtained weekly. A single oral dose of 75 mg/m^2 (100% 6MP) was given prior to breakfast on day 0 and 3 ml blood samples were obtained for 6MP and TGN assay at 4, 48, 96, and 168 hrs post-dose. Doses 2, 3, and 4 were given on days 7, 14, and 21 with the monitoring of 6MP and TGNs as in the week 1 schedule.

The 100% weekly 6MP study was followed by 10% 6MP daily. If the neutrophil or platelet counts fell below 1.0 or 100 × 10^9/1 respectively, the dosage was reduced to 10% on alternate days. If the neutrophil count or platelet count fell to < 0.5 or < 50 × 10^9/l respectively, the drug was withdrawn. 6MP and TGN concentrations were monitored at the time of the weekly blood cell count (pre

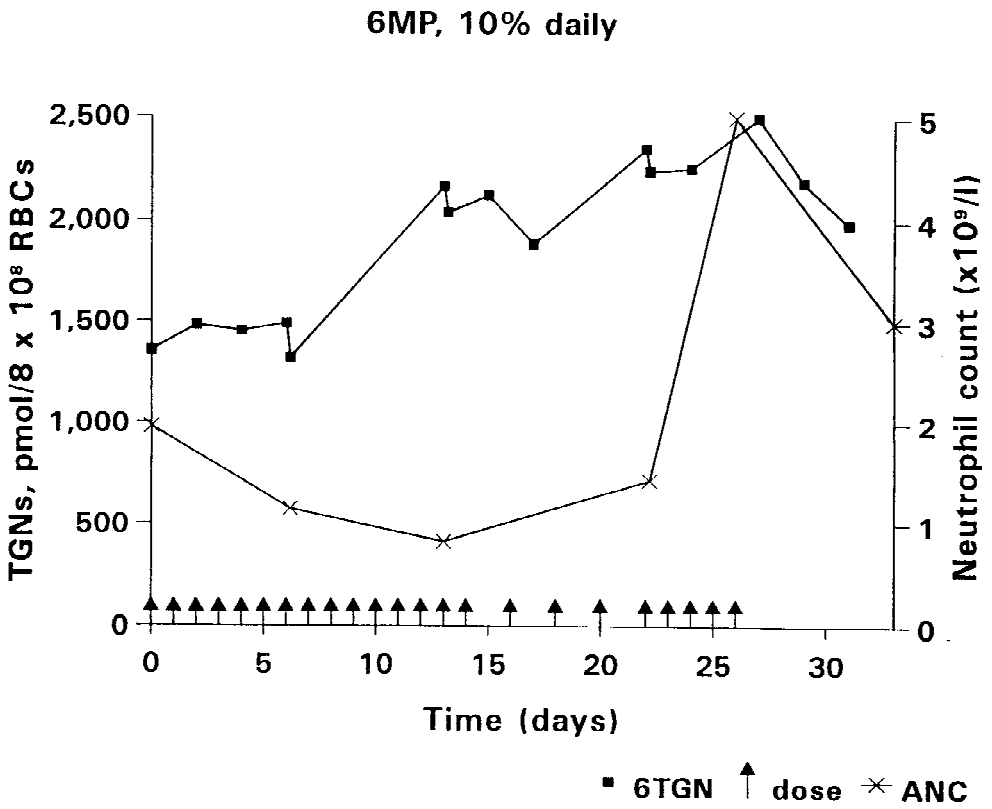

Fig. 2. The accumulation of RBC TGNs during the 10% (7.5mg/m^2) daily dosage study. Daily 6MP was taken, as indicated by the small arrows. The TGNs measured in the day 0 sample reflect that remaining 15 days after the fourth 100% weekly 6MP dose. The Figure illustrates the neutrophil (ANC) counts on days 0, 6, 13, 22, 26, and 33. The platelet counts on those days were 193, 144, 215, 221, 201, and 130 × 10^9/l respectively. Diarrhoea and vomiting necessitated drug withdrawal on day 26.

and at 4 hr post-dose) and at 4 hrs post-dose at all other times. All blood samples were obtained via a central venous catheter.

RESULTS

The accumulation of RBC TGNs during the weekly dosing schedule are illustrated in Fig 1. The pre-dose blood sample contained low levels of TGNs (34 pmol/8 × 10^8 RBCs), 10 weeks after the previous 6MP cycle. TGNs increased steadily in the 7 days following each 6MP dose. The TGNs continued to rise after the last dose. After the 100% dosage study the child remained well.

6MP was commenced at 10% (Fig 2). The second week of 10% dosing saw an increase in TGNs towards the maximum values observed during weekly 100% therapy, and neutropenia occurred on day 14. Alternate day therapy was given for 8 days, but five days after readjustment to 10% daily dosages diarrhoea and vomiting necessitated drug withdrawal. A weight loss of 1.5 kg occurred over this period. There was no evidence of liver function abnormalities or jaundice. The TGN half-life, calculated on drug withdrawal, was 12 days.

Analysis of plasma drug levels [11] showed that during the weekly dosage study the 4 hr post-dose samples contained 6MP (range 96–148 pmol/ml plasma). In all other blood samples, 6MP was below the lower limit of detection (30 pmol/ml plasma).

Subsequent Antimetabolite Therapy

6MP was restarted at 10% daily. Methotrexate was restarted at 20 mg/m^2 weekly (100% protocol dose) and prescribed as per protocol. Over the next 7 weeks the drugs were withdrawn for 2 weeks because of thrombocytopenia, nausea, and continued bowel problems (an increased frequency of stools up to 4 per day and weight loss). 10% alternate day dosage was introduced. After 15 weeks alternate day 6MP TGNs were 1670 pmol/8 × 10^8 RBCs. The child continues in her first remission 8 months after completing therapy, 2.7 years post diagnosis.

DISCUSSION

6MP metabolism was studied during two attenuated dosing regimens. Previous schedules reporting controlled

myelosuppression at 30 mg/m^2 twice weekly [8] prompted us to investigate TGN formation during weekly dosing at 75 mg/m^2 (100% protocol) and compare this to a 10% daily dosing schedule, the latter of which we had experience of in the TPMT deficient child [9]. The TPMT deficient child reported in this paper had accumulated 481 pmol TGNs 7 days after a single oral 6MP dose of 75 mg/m^2. After 4 doses, given at weekly intervals, TGNs increased nearly 5-fold to peak at 2348 pmol compared to a range of 113–1340 pmol, median 284, measured in children with TPMT activity receiving 75 mg/m^2 6MP daily [1]. Throughout the continued accumulation of RBC TGNs plasma levels of 6MP were low or absent.

The half-life of RBC TGNs was biphasic and measured in weeks, thus the continued production of TGNs days after taking the weekly 100% dose raised the possibility of unregulated exposure to cytotoxic metabolites. Unacceptable gastrointestinal symptoms were present throughout the 10% study. Adjustment to 10% alternate day treatment eliminated these apparently toxic effects and maintained TGNs at 1670 pmol, just above the upper limit of TPMT active children [1], and similar to TGN concentrations measured in children with ALL receiving 6-thioguanine as an alternative thiopurine to 6MP [13]. For comparison, other attenuated dosage regimens for the TPMT deficient child have explored 10 mg/m^2 6MP 3 times per week (TGN maximum = 1043 pmol) [7], 30 mg/m^2 twice weekly (1059 pmol TGNs after 5 weeks) [8], 30 mg/m^2 3 times weekly (2252 pmol TGNs after 7 weeks) [8], and 7.5 mg/m^2 daily (1701 pmol TGNs after 6 weeks) [9].

The aim of treatment with 6MP in the UK ALL protocols is controlled myelosuppression. In the patient reported here this was best achieved at 10% of the protocol standard dose (7.5 mg/m^2) with alternate day therapy. Of 400 children developing ALL in the UK each year, one or two will have TPMT deficiency, and failure to recognise such children will result in unnecessary reductions in other maintenance treatment. To pick up such children the measurement of TPMT activity at diagnosis is probably justified. An alternative is to monitor RBC 6TGN concentrations after the start of 6MP therapy. Mutant alleles have now been characterised from TPMT deficient individuals [4,14], permitting the eventual development of genotype tests for this important genetic polymorphism.

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